Biologically Active Terpenoids from *Tamarix* Species

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Abstract

We carried out research of bioactive substances obtained from ethanol extract (70%). This ethanol extract (70%) was obtained by us from the aerial parts of *Tamarix* plants (*T.laxa* Willd., *T.hispida* Willd., *T.ramosissima* Ledeb. and *T.elongata* Ledeb.). These plants belong to the family of *Tamaricaceae* which grow on the territory of Kazakhstan. For the preliminary separation of bioactive substances, the fractional extraction of ethanol extracts (70%) of *Tamarix* species was carried out using chloroform and ethyl acetate. Chloroform and ethyl acetate extracts contained terpenoids (positive reactions with Lieberman-Burchard reagent and cerium sulfate).

Isolation of terpenoids from chloroform and ethyl acetate extracts was achieved by a series of separations on chromatography column over silica gel and these terpenoids were eluted with gradient mixture of hexane-ethylacetate (0-100%). From the aerial parts of *Tamarix* (*T.laxa* Willd., *T.hispida* Willd., *T.ramosissima* Ledeb., *T.elongata* Ledeb) five terpenoids were isolated. Their structures of 1-5 were found using alkaline hydrolysis and UV, IR, ^{1}H and ^{13}C NMR, COSY-45°, HMQC, HMBC, EIMS, FABMS (-ve) spectral analyses.

Pentacyclic triterpenoids: ursolic acid (1), 3β-al-D- friedoolean-14-en-28-oic acid methyl ether (2), 3-*α*-[3´´,4´´-dihydroxy-trans-cinnamoyloxy]-D-friedoolean-14-en-28-oic acids (isotamarixen) (3), 3-*α*-hydroxy-D-friedoolean-14-en-28-oic acid (4) and 3-*α*-[4´´-dihydroxy-trans-cinnamoyloxy]-D-friedoolean -14-en-28-oic acid (5) were isolated from the aerial parts of *Tamarix*.

Ethanol extracts (70%), chloroform, ethylacetate extracts from *Tamarix laxa*, *Tamarix elongata* showed antioxidant activity. Antioxidant activities were determined using the DPPH (1, 1-diphenyl-2-picrylhydrazyl radical) scavenging method.

Ethanol extracts from *Tamarix hispida* (50%) were found to contain the triterpenoid: 3-*α*-[3´´, 4´´-dihydroxy-trans-cinnamoyloxy]-D-friedoolean-14-en-28-oic acids (isotamarixen), which were tested *in vitro* for their effect on the α-glycosidase enzyme activity.

3-*α*-[3´´, 4´´-dihydroxy-trans-cinnamoyloxy]-D-friedoolean-14-en-28-oic showed inhibiting effect on the *α*-glycosidase enzyme activity and was found to be a potent antioxidant.

Introduction

Plants from *Tamarix* genus of *Tamaricaceae* family are used in traditional medicine in the treatment of dysentery and ulcers [1] for example four terpenes: *β*-amyrin, D-fridoolean-14-en-3α-28-diol (isomyricadiol), 28-hydroxy-D-fridoolean-14en-3-one, lupeol, myricadiol, tamarixol, tamarixone, ursolic acid have been isolated from the species of *Tamarix* (*T. troupii*, *T.gallica*, *T.aphyl* and *T.chinensis*) [2].

Terpenoids represent a large group of organic compounds containing isoprene structure. The variety of properties, structural features, determines their wide range of biological action as antitumor, antimicrobial, antiviral and immunopotentiating activity for example ursolic acid and pentacyclic triterpene acid, are often-met as a constituents of medicinal herbs in the plant kingdom. Ursolic acid has been reported to display remarkable biochemical activities to influence processes that are deregulated during cancer development. These include inhibition of tumorgenesis, tumor promotion, invasion,

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metastasis, angiogenesis and induction of tumor cell differentiation. In addition, Ya-Ling Hsu et al. have reported that ursolic acid inhibited the cell proliferation of humans lung cancer cell line A549 and provided a molecular understanding for this effect [3].

Two new taraxasterane-type triterpenes: 20*β*, 28-epoxy-28*α*-methoxytaraxasteran-3*β*-ol and 20*β*, 28-epoxytaraxaster-21-en-3*β*-ol, were isolated from ethyl acetate extract of the leaves of *Nerium oleander*, together with ursane-type triterpenes: 28-nor-urs-12-ene-3*β*,17*β*-diol and 3*β*-hydroxyurs-12-en-28-aldehyde and they have anti-inflammatory activity [4] for example, four new triterpenoids: 6*β*-hydroxy-3-oxo-11*α*,12*α*-epoxyolean-28,13*β*-olide, 3*β*,6*β*-dihydroxy-11α,12*α*-epoxyolean-28,13β-olide, 3*β*,6*β*-dihydroxy-11-oxo-olean-12-en-28-oic acid and 3*β*-hydroxy-12-oxo-13H*α*-olean-28,19*β*-olide were evaluated for cytotoxicity against the P388 (murine lymphocyte leukemia) and the A549 (human lung cancer) cell lines [5].

Besides, more attention is also paid to the study of the content of antioxidants in medicinal plants. For the inhibition of oxidation processes you can use different synthetic and natural antioxidants [6]. The use of synthetic antioxidants restricted because of their toxicity, therefore natural herbal antioxidants are very important.

An antioxidant may be defined as «a substance which can prevent or inhibit the oxidation of autooxidizable materials in living cells» [7]. Antioxidants are the compounds which can retard aging, cancer and heart diseases in humans by inhibiting or quenching free radicals and reactive oxygen species, also they can increase the shelf life and quality of food and oils [8].

In 2000 171 million cases of diabetes mellitus disease were registered. It is expected that in 2030 this number may be about 366 million. Two types of diabetes mellitus are known. There are two types of diabetes – insulin-dependent diabetes mellitus and non-insulin-dependent diabetes mellitus (also known as Type II or maturity-onser). To reduce the risk of chronic diseases antioxidants should be used. *α*-glucosidase and *α*-amylase enzymes play an important role in the treatment of diabetic patients and borderline patients. Many herbal extracts have been reported to have anti-diabetic activity. The medicinal plants or natural products involve the retarding of the absorption of glucose by inhibiting the carbohydrate hydrolyzing enzymes. Several *α*-amylase inhibitors including acarbose, voglibose and miglitol are clinically used for treatment but they are expensive and clinical side effects take place when we use them. «Hence screening of *α*-glucosidase inhibitors from plants and synthetic sources are increasing and inhibitors of these enzymes have been recently developed from natural sources» [9].

Terpenoids also have an inhibitory effect and control of glucose in blood, for example, two new triterpenoids: 18*α*,19*β*-20(30)-taraxasten-3*β*,21*α*-diol (Cichoridiol) and 17-*epi*-methyl-6-hydroxy-angolensate (Intybusoloid) were obtained from the methanolic extract of the seeds of *Cichorium intybus*. They showed a good *α*-glucosidase inhibitory activity [10].

Although the number of effective tools for the isolation from plants were established based on terpenoids, it is important to find ways for isolation. Recently, when reviewing the literature on the genus of *Tamarix* we have found researchers from Pakistan, India and China who isolated a number of terpenoid compounds. But we were the first who started to study the biological activity of these classes of compounds and isolate them from the plant species that grow in Kazakhstan.

Experimental

Materials and Methods

The objects of the study - plants of *Tamarix* genus - *Tamarix laxa* Willd., *Tamarix hispida* Willd., *Tamarix ramosissima* Ledeb., *Tamarix elongata* Ledeb., belonging to the family of Tamaricaceae. These plant species were collected in Almaty region, the Republic of Kazakhstan.

For the study of qualitative composition of the extracts, as well as the individual compounds the methods of thin-layer chromatography - TLC (Silica gel DC - Alugram 60 UV 254 MERCK art. 7739); paper chromatography - Watman S2 paper made in Germany.

Systems of solvents:

I. Benzene: acetic acid : water (6:7:35)

II. Hexane: chloroform (9:1)

III. Dichloromethane: methanol (9.7:0.3)

For the visualization of the following reagents: UV - light, cerium sulfate, Lieberman-Burchard reagent were used. Colum chromatography was performed over silica gel 60, 70-230 mesh (MERCK). Solvent system for column chromatography: hexane – ethylacetate (0-100%) was used.

Alkaline hydrolysis was carried out in 3% potassium hydroxide solution in ethanol at 60ºC for 2 hours.

Melting point was defined using Buchi 535 device in glass capillaries and in Kophler block; optical rotation - on JASCO DIP 360 polarimeter.

UV and IR spectra were recorded on Hitachi UV 3200 and JASCO 302-A spectrophotometers. EIMS,

FABMS (-ve) was measured on Varian MAT 311A and JEOL HX 110 mass spectrometers. The NMR (^{1}H, ^{13}C NMR, COSY-45˚, HMQC, HMBC) were recorded on Bruker AMX 500 MHz NMR spectrometers.

Antioxidant activity: The tested samples were prepared by dissolving the compound by DMSO. 316 μM solution of DPPH was prepared in ethanol and the mixtures reagents of containing 5 μl of tested samples and 95 μl of DPPH (final conc. of test samples 1 mM and 300 μM of DPPH) in 96 μl well microtiter plates were incubated at 37ºC for 30 minutes and the absorption was measured at 515 nm. Percent inhibitions were determined by the comparison with a DMSO treated control group [7].

α-glycosidase enzyme activity. The *α*-glucosidase (E.C.3.2.1.20) enzyme inhibition assay was performed according to the slightly modified method developed by Oki et al. The inhibition was measured spectrophotometrically at pH 6.9 and at 37 °C using 0.5 mM *p*-nitrophenyl R-D-glucopyranoside (PNP-G) as a substrate and 250 m units/mL enzyme, in 50 mM sodium phosphate buffer containing 100 mM of NaCl. Acarbose (0.78 mM) was used as a positive control. The increment in absorption at 400 nm, due to the hydrolysis of PNP-G by R-glucosidase, was monitored continuously spectrophotometrically (Spectra Max, Molecular Devices, CA) [11].

The biological activities were investigated in the laboratories of the H.E.J. Research Institute of Chemistry International Center for Chemical and Biological Sciences University of Karachi, Pakistan.

Results and Discussion

Plant raw material (3 kg) was extracted using ethanol extract (70%) at room temperature for continuosly 72 hours. After filtration of the combined aqueous-alcoholic extract, ethanol extract (70%) was concentrated under mild conditions in the vacuum when the temperature of the water bath was not more than 45ºC. Aqueous concentrate was subjected to sequential fractionation using chloroform and ethyl acetate respectively. The chemical compositions of chloroform, ethyl acetate and aqueous extracts, were investigated using the methods of TLC which showed the presence of terpenoids, alcohols, sterols, chlorophylls while, triterpenoids and flavonoids were found in ethyl acetate extract.

Chloroform and ethyl acetate extracts of the investigated species were concentrated then separated on chromatography column with silica gel and the mixture of hexane- ethyl acetate as a solvent with increasing gradient polarity. In the first fractions, lipophilic components were separated, and subsequent mixture of terpenoids was obtained from the subsequent fractions, the mixture was subjected to the repeated chromatography column using silica gel. As a result we obtained the substances 1-5 which were in individual state. The isolated substances belonged to triterpenoids by the positive reactions with Lieberman-Burhard reagent method and cerium sulfate [12].

The structure of the separated substances 1-5 were identified by chemical (alkaline hydrolysis) and spectral (UV-, IR-, ^{1}H and ^{13}C NMR (BB, DEPT), COSY-45˚, HMQC, HMBC, EIMS, FABMS (-ve) methods of analysis.

Substance 1 was identified as: ursolic acid previously isolated from other plant species in Tamarix genus [1].

Substance 2 was related to pentacyclic triterpenoids because of claret coloring using cerium sulfate and Lieberman-Burchard reagent and reaction. In IR spectrum there were absorption bands which were typical for methyl, methylene and ketonic groups in the range of 2864-2933 cm^{-1} and 1689 cm^{-1}, respectively. In the mass spectrum of molecular ion the peak *m/z* 482 corresponding to $C_{32}H_{50}O_3$ molecular formula was registered by the means of EIMS method. Fragmentation with *m/z* 438 corresponded to the detaching of aldehyde group in C-3 position and the detaching of methyl group from ester in C-17 position. Fragments with *m/z* 248 ($C_{16}H_{23}O_2$), 204 ($C_{15}H_{23}$), 189 ($C_{14}H_{21}$) and 133 ($C_{10}H_{12}$) corresponded to retro-dien disintegration on Diels-Alder, which is typical for pentacyclic triterpenoids.

Also, 32 signals of carbon atoms were observed in a spectrum of NMR ^{13}C. By the means of DEPT in a normal phase seven methyl (C-23-27, C-29-30), four methyne (C-5, C-9, C-15, C-18), and in a return phase ten methylene carbon atoms (C-1, C-2, C-6, C-7, C-11, C-12, C-16, C-19, C-21, C-22) were registered. Proton signals of seven methyl groups in a spectrum ^{1}H NMR were registered at δ 0.82-0.98 as 3H singlets, and twenty protons of methylene groups were registered as multiplet signals at δ 1.08-1.98.

Besides, in ^{1}H NMR spectrum at δ 5.54 olefinic proton was registered in a double bond H-15 (1H, dd, J_1=11.0 and J_2=3.4 Hz), indicating that substance 2 belongs to taraxeran-14-en type or to D-fridoolean derivatives, and at δ 3.30 it belongs to the β form.

In ^{13}C NMR spectrum signals of carbon atoms with a double bond (C-14 and C-15) were registered at δ 161.0 and δ 117.0; signals C-28 and C-3 were registered at δ 179.0 and δ 76.0, respectively. Singlet at δ 3.80 in a spectrum ^{1}H NMR and the region of δ 56.0 in a spectrum ^{13}C NMR are typical for

the methyl ether of carbonyl group at C-28, but in ^{13}C NMR spectrum of the substances 2 an additional signal of aldehydic carbon atom was found out at δ 238.0. COSY-45˚ and HMBC spectroscopy were used to find the presence of the position of the double bond of $COOCH_3$, CHO and CH_3 groups. Carbon atom of C-15 in the HMBC spectrum interacts with protons H-16, H-18, and C-14 with protons at C-26, C-18. The COSY-45˚ spectrum also confirms the interaction of H-15 and H-16 protons. Hence, according to 2D spectra the double bond is located between C-14 and C-15 atoms.

Position of $COOCH_3$ group was confirmed in the spectrum HMBC: signals of protons H-18, H-16 and H-22 interact with carbon atom in C-28 and C-17 position. Carbon atom of aldehydic group interact with a methyl proton at C-23 (δ 0.98), that confirms addition of the aldehydic groups in C-3 position. Besides, in the ^1H NMR spectrum the doublet of doublets signal with J_1=9.0, J_2=4.7 Hz, indicates the fact that the methyne proton at C-3 is in an axial, and aldehydic group is in an equatorial position. The positions of methyl ether were completely established by the HMBC spectrum. On the basis of the methods of the physical and chemical analyses of the structure for the substance 2 was identified as methyl ether of the 3-*β*-al-D-fridoolean-14-en-28 carboxylic acid.

Substance 2 was identified as 3*β*-al-D- friedoolean-14-en-28-oic acid methyl ether. Physicochemical characteristics of the substance 2 are shown in the Table 1 and Fig. 1. The substance 2 is a new chemical compound which was isolated from *T.laxa* and *T.elongata* for the first time [13]. We found this substance 2 in the *T.hispida* and *T.ramosissima.*

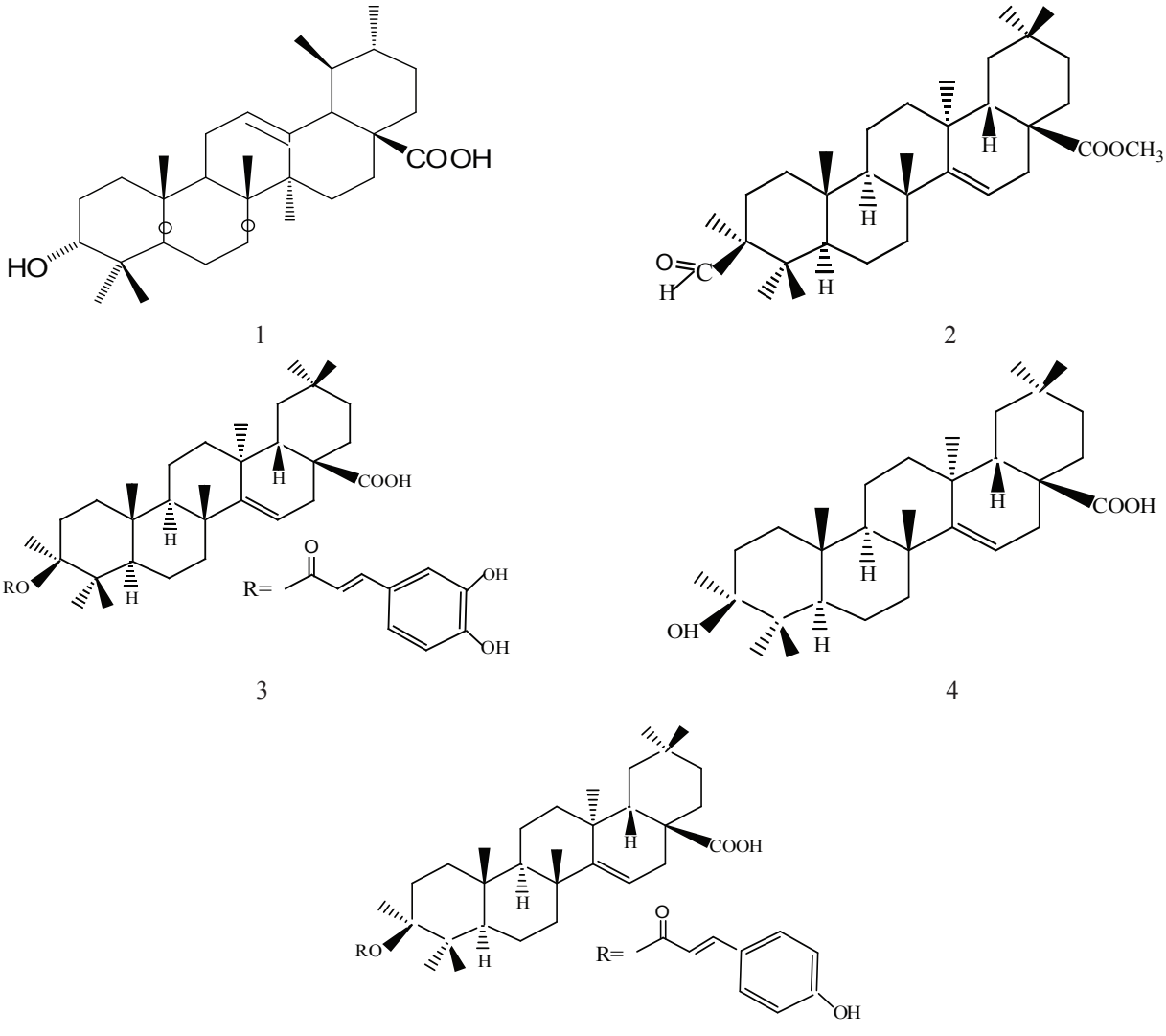

Fig. 1. Structures of terpenoids from genus *Tamarix*.

Table 1
Physico-chemical characteristics of terpenoids isolated from genus *Tamarix*

№	Substance	Characteristics
1	ursolic acid - 1	$C_{30}H_{48}O_3$, white crystals with m.p. - 286-288ºC (from hexane). IR (KBr, ν_{max}, cm^{-1}): 3400, 2943, 2864, 1692, 820. ^1H NMR ($CDCI_3$, 500 MHz): 5.20 (^1H, t, J=3.5, H-12), 3.20 (1H, dd, J=10.0 5.2, H-3), 2.10 (1H, d, J=11.5, H-18), 2.01-1.15 (m, 22H), 1.12 (s, 3H, C-23), 0.97 (s, 3H, C-27), 0.95 (s, 3H, C-26), 0.92 (s, 3H, C-24), 0.86 (d, 3H, C-29), 0.85 (d, 3H, C-30), 0.75 (s, 3H, C-25).
2	3*β*-al-D-friedoolean-14-en-28-oic acid methyl ether - 2	$C_{32}H_{50}O_3$, white crystals with m.p. 292-294ºC (from ethanol), R_f 0.65 (II). IR (KBr, ν_{max}, cm^{-1}): 2933, 2864, 1689, 1632, 1601. EIMS *m/z*: 482 (20.4), 438 (4.2), 248 (25.7), 204 (23.1), 189 (100.0), 133 (29.7). ^1H NMR (CD_3COCD_3, 500 MHz): 3.80 (c,$COOCH_3$), 1.30 (2H, m, H-1), 1.98 (2H, m, H-2), 3.30 (1H, dd, J=9.0, 4.7, H-3), 1.35 (1H, m, H-5), 1.46 and 1.65 (2H, m, H-6), 1.38 and 1.91 (2H, m, H-7), 1.53 (1H, m, H-9), 1.54 and 1.66 (2H, m, H-11), 1.65 and 1.70 (2H, m, H-12), 5.54 (1H, dd, J=11.0, 3.4, H-15), 1.98 and 2.37 (2H, m, H-16), 2.37 (1H, m, H-18), 1.10 and 1.27 (2H, m, H-19), 1.08 and 1.65 (2H, m, H-21), 1.48 and 1.65 (2H, m, H-22), 0.98 (3H, s, H-23), 0.82 (3H, s, H-24), 0.89 (3H, s, H-25), 0.90 (3H, s, H-26), 0.92 (3H, s, H-27), 0.93 (3H, s, H-29), 0.94 (3H, s, H-30). ^{13}C NMR_(CD_3COCD_3, 125 MHz): 36.1 (C-1), 23.2 (C-2), 76.0 (C-3), 38.1 (C-4), 51.3 (C-5), 19.3 (C-6), 42.2 (C-7), 39.9 (C-8), 49.8 (C-9), 36.1 (C-10), 18.0 (C-11), 33.8 (C-12), 38.0 (C-13), 161.0 (C-14), 117.0 (C-15), 32.6 (C-16), 52.0 (C-17), 42.5 (C-18), 34.5 (C-19), 32.4 (C-20), 34.3 (C-21), 31.3 (C-22), 27.0 (C-23), 22.5 (C-24), 15.7 (C-25), 26.4 (C-26), 22.6 (C-27), 179.0 (C-28), 31.6 (C-29), 30.0 (C-30), 56.0 ($COOCH_3$), 238.0 (CHO).
3	3-*α*-[3′′, 4′′-di-hydroxy-trans-cinnamoyloxy]-D-friedoolean-14-en-28-oic acids (isotamarixen) - 3	$C_{39}H_{54}O_6$, white amorphous substance with m.p. 196-198ºC (from ethanol), $[\alpha]_D^{22}$ -22 (C. 0.04; methanol), R_f 0.35 (III). UV (λ_{max},CH_3OH, nm): 216, 244, 255, 305, 372. IR (KBr, ν_{max}, cm^{-1}): 3411, 1269, 1691, 1600, 812. EIMS *m/z*: 617 [M-1] (20), 437 (15), 247 (8), 206 (5), 188 (90), 132 (5). ^1H NMR (CD_3OD-$CDCI_3$, 500 MHz): 1.30 (2H, m, H-1), 1.95 (2H, m, H-2), 4.69 (1H,t, J=2.5, H-3), 1.35 (1H, m, H-5), 1.40 and 1.60 (2H, m, H-6), 1.40 and 1.95 (2H, m, H-7), 1.55 (1H, m, H-9), 1.55 and 1.65 (2H, m, H-11), 1.60 and 1.76 (2H, m, H-12), 5.57 (1H, dd, J_1=3.5, J_2 =8.0, H-15), 1.96 and 2.37 (2H, m, H-16), 2.37 (1H, m, H-18), 1.10 and 1.24 (2H, m, H-19), 1.05 and 1.6 (2H, m, H-21), 1.5 and 1.7 (2H, m, H-22), 0.87 (3H, s, H-23), 0.92 (3H, s, H-24), 0.97 (3H,s, H-25), 0.98 (3H, s, H-26), 0.94 (3H, s, H-27), 0.96 (3H, s, H-29), 0.93 (3H, s, H-30), 6.26 (1H, d, J=16.0, H-21), 7.51 (1H, d, J=16.0, H-3′), 7.03 (1H, d, J=2.0, H-21), 6.76 (1H, d, J=8.0, H-5′′), 6.92 (1H, dd, J_1=2.0, J_2=8.0, H-6′′).
4	3-*α*-hydroxy-D-friedoolean-14-en-28-oic acid - 4	$C_{30}H_{48}O_3$ white amorphous substance with m.p. 270-272ºC (from chloroform), $[\alpha]_D^{22}$ –13.50 (C. 0.04; chloroform), R_f 0.20 (III). IR (KBr, ν_{max}, cm^{-1}): 3448, 1690, 1460, 1386, 1061, 993. EIMS *m/z*: 456 (5), 438 (3), 248 (49), 207 (30), 189 (100), 133 (26); 438 [M-H_2O]. ^1H NMR (CD_3OD-$CDCI_3$, 500 MHz): 1.28 (2H, m, H-1), 1.92 (2H, m, H-2), 4.41 (1H,s, $W_{1/2}$=2.56, H-3), 1.34 (1H, m, H-5), 1.40 and 1.60 (2H, m, H-6), 1.40 and 1.90 (2H, m, H-7), 1.53 (1H, m, H-9), 1.53 and 1.64 (2H, m, H-11), 1.60 and 1.73 (2H, m, H-12), 5.53 (1H, dd, J_1=3.5, J_2 =8.0, H-15), 1.90 and 2.35 (2H, m, H-16), 2.35 (1H, m, H-18), 1.10 and 1.22 (2H, m, H-19), 1.02 and 1.55 (2H, m, H-21), 1.5 and 1.7 (2H, m, H-22), 0.85 (3H, s, H-23), 0.90 (3H, s, H-24), 0.95 (3H, s, H-25), 0.97 (3H, s, H-26), 0.93 (3H, s, H-27), 0.95 (3H, s, H-29), 0.92 (3H, s, H-30).
5	3-*α*-[4′′-hydroxy-trans cinnamoyloxy]-D-friedoolean-14-en-28-oic acid - 5	$C_{39}H_{54}O_5$, white amorphous substance with m.p. 245-247ºC (from chloroform). IR (KBr, ν_{max}, cm^{-1}): 3448, 2935, 2865, 2366, 1689, 1601, 1460, 1211, 993, 766. ^1H NMR ($CDCI_3$, 500 MHz): 7.52 (2H, d, J=8.0, H-2′′, H-62); 6.87 (2H, d, J=8.0, H-3′′, H-5′′), 7.58 (1H, d, J=16.0, H-3′); 6.30 (1H, d, J=16.0, H-2′), 5.57 (1H, dd, J=8.0, 3.5, H-15); 4.71 (1H, t, J=2.5, H-3), 0.87 - 0.98 (3H, s, 7 CH_3), 1.30-2.37 (m, CH_2 and CH).

Substances 3 and 4 were identified as a 3-*α*-[3´´4´´-dihydroxy-trans-cinnamoyloxy]-D-friedoolean-14-en-28-oic acids (3) and 3-*α*-hydroxy-D-friedoolean-14-en-28-oic acid (4), respectively.

Substance 3 was isolated as an amorphous solid with IR absorbtions at 1259 cm^{-1} (*α, β*-unsaturated ester) and 3411 cm^{-1} (hydroxy), the bands of absorption in the region of 1691 cm^{-1} show the presence of ester group. These data are given in the Table 1. The UV spectrum showed absorptions at 216, 244, 255,305 and 372 nm. The 1H NMR signals of seven 3H singlets at δ=0.87-0.98 were the seven tertiary methyls. An olefinic proton appeared as a double doublet at δ 5.57 (1H, dd, J=8.0 Hz, J=3.5 Hz, H-15). The chemical shift, multiplicity, coupling constants of the olefinic proton and chemical shifts of the tertiary methyls signals were in the agreement with those of taraxer-14-en skeleton.

The H-3 (δ=4.69) was proved to be in a *β* conformation from the coupling constant (J=2.5 Hz) of its signal. The coupling relationship between aromatic proton signals, appeared at δ=7.03 (1H, d, J=2.0 Hz, H-2``), 6.76 (1H, d, J=8.0 Hz, H-5``) and 6.92 (1H, dd, J=8.0 Hz, J=2.0 Hz, H-6``). 1, 3, 4-trisubstituted aromatic ring was found. Two 1H doublets at δ=6.26 and 7.51 (J=16.0 Hz) represented the trans-substituted olefinic H-2` and H-3, which indicated the presence of O-3``, 4``-dihydroxycinnamoyl group. The α-position of dihydroxycinnamoyl group at C-3 was found by the small $^{3}J_{HH}$ (2.5 Hz) of the equatorial H-3 signal. The ^{13}C NMR spectrum showed seven methyls, ten methylenes, ten methin and twelve quaternary carbon signals. The signals for eight olefinic carbons (C-2`, C-3`, C-1``, C-2``, C-3``, to C-6``), an ester carbonyl (C-1`), carbonyl (C-1`), a carboxyl carbon (C-28) and one double bond of carbon (C-14 and C-15) were registered at δ=115.5, 146.5, 127.4, 115.0, 146.4, 149.2, 117.7, 122.7, 168.0, 182.2, 161.3, and 116.2, respectively. The position of the COOH-moiety at C-17 was found by a quaternary carbon signal at δ=52.1 which was assigned to C-17. The HMBC long-range coupling, data in which H-18 exhibited an interaction with C-28 (δ=182.2), further confirmed the location of COOH at C-17. The H-3 (δ=4.69) showed a long-range correlation with the C-1` (δ=168.0), indicating that the 3``, 4``-dihydroxycinnamoyl group was located at C-3. To confirm the position of cinnamoyl moiety, basic compound 3 was connected with it. The EIMS of 3 showed the highest peak at *m/z*=438.3507 corresponding to the formula $C_{30}H_{46}O_2$. The FABMS (-ve) showed the pseudomolecular ion at *m/z*=617.432 corresponding to the molecular formula $C_{39}H_{53}O_6$. The mass difference between EIMS data was due to the loss of a 3``, 4``-dihydroxycinnamoyl group ($C_9H_7O_4$). The characteristic fragments at *m/z*=248, 203, 189 and 133 resulting from the retro-Diels-Alder cleavage presumed a taraxeer-14-ene skeleton with a carboxylic group. On the basis of all spectral observations, compound 3 was found to be 3-α-[3´´,4´´-dihydroxy-trans-cinnamoyloxy]-D-friedoolean-14-en-28-oic acids. The substance 3 is a new chemical compound. Physico-chemical characteristics and structures are given in the Table 1 and in Fig. 1. The substance 3 is a new chemical compound which was isolated from *T.hispida* for the first time [14]. We found this substance 3 in the *T.laxa*, *T.elongata* and *T.ramosissima*.

The compound 4 is the hydrolyzed product of compound 3 in EIMS of (*m/z* $[M]^{+}$=456) which corresponds to the molecular formula $C_{30}H_{48}O_3$. In the ^{1}H NMR spectrum of compound 4, H-3 appeared as a broad singlet at δ=4.41 ($W_{1/2}$=2.56 Hz) which indicated that H-3 was equatorially oriented. A triterpene, 3α-acetyltaraxer-14-en-28-oic acid, previously isolated from *Phytolacca acinosa* [15] is structurally related to 3 except that the 3-α-acetyl group is present at C-3. Physico-chemical characteristics and structures are given in the Table 1 and in Fig. 1.

Substance 5 is derived from 3, as a result of hydrolysis we obtained a glycone 4, which was identified by us in the hydrolysate. Then we compared a glycone 4 with reliable sample of *p*-cumaric acid with R_f 0.33 (I). This substance gave a positive Lieberman-Burchard reaction. It had a molecular formula of $C_{39}H_{54}O_5$ by EIMS. Comparison of its ^{1}H and ^{13}C NMR data with those of 5 had the same triterpenoid moiety, the only difference was the substitution group of C-3. The ^{1}H NMR spectrum of 5 showed aromatic proton signals at δ 7.52 (2H, d, J=8.7Hz) and 6.87 (2H, d, J=8.7Hz), characteristic of an AABB system, which were compatible with the presence of a p-hydroxybenzoyl moiety, and the olefinic proton signals at δ7.58 (1H, d, J= 16.0 Hz) and 6.30 (1H, d, J=16.0 Hz). They were identical to those of trans-cinnamoyl. The structure of 5 was found to be 3-*α*-[4´´-hydroxy-trans-cinnamoyloxy]-friedoolean -14-en-28-oic acid, which was previously separated from *Miricaria* genus (*Tamaricaceae*) [16]. Physico-chemical characteristics and structure of the substance 5 are given in the Table 1 and in Fig. 1. These substance 5 were identified and characterized by us in the plants of *T.hispida*, *T.ramosissima*, *T.laxa* and *T.elongata*.

Antioxidant Activity

The different ethanol extracts (70%), ethyl acetate extracts and chloroform extracts obtained from the plants of *T.laxa* and *T.elongata* were studied for

the antioxidant activity using the DPPH (1, 1-diphenyl-2-picrylhydrazyl radical) scavenging method [7]. Propyl gallate was used as a control. The results presented in Table 2 showed that the antioxidant activity of ethanol extract (70%) from *T.elongata* and chloroform extractis from *T.laxa* greater than the antioxidant activity of the control. The antioxidant activity of ethyl acetate extracts was found to be similar to the antioxidant activity of the control.

Table 2
Antioxidant activity of extracts from *Tamarix laxa* and *Tamarix elongata*

Extracts/ Control	Concentration in μg/ml	Inhibition (%)
Tamarix laxa		
ethanol extract (70%)	200	87.11
ethyl acetate extract	200	92.10
chloroform extract	200	95.30
Tamarix elongata		
ethanol extract (70%)	200	96.01
ethyl acetate extract	200	91.76
chloroform extract	200	56.97
Propyl gallat	200	92.00

Substance 3 which was isolated from all *Tamarix* species showed also antioxidant activity which was approximately equal to the control (propyl gallat). The data of biological activity is shown in Table 3.

Table 3
Antioxidant activity of isotamarixen

Substance/Control	IC_{50} (μM)
Isotamarixen	29±0.5
Propyl gallat	30±0.27

Inhibition of α-glycosidase Enzyme

α-glycosidase enzyme was involved in the inhibition of elevated glucose level in blood thus preventing diabetes [9, 11]. From Tables 4 and 5 we found that the ethanol extracts (50%) of *T.hispida* at concentration 30 μM showed high α-glycosidase enzyme inhibitory activity (92.50%) that is at the level with the control (acarbose), also substances 3 at concentration of 0.00152 μM and 0.000781 μM showed high inhibitory activity of α-glycosidase enzyme by 99% and 94% respectively.

Finally, the results indicate that terpenoid-3-α-[3′′, 4′′-dihydroxy-trans-cinnamoyloxy]-D-friedoolean-14-en-28-oic acids (isotamarixen) showed sufficiently inhibiting effect on the α-glycosidase enzyme and have high antioxidant activity.

Table 4
α-glycosidase enzyme activity of extract from *T. hispida*

Extract/Control	Concentration in μM	Inhibition (%)
ethanol extract (50%)	30	92.37
Acarbose	30	100

Table 5
α-glycosidase enzyme activity of isotamarixen

Substance/ Control	Concentration in μM	Inhibition (%)
Isotamarixen	0.001562	99
	0.000781	94
	0.000390	69
	0.000195	31
	0.000097	23
Acarbose	(780 ± 28) μM	100

Conclusions

Five terpenoids were isolated from chloroform, ethylacetate extracts of aerial parts of *Tamarix – T. laxa* Willd., *T. hispida* Willd., *T. ramosissima* Ledeb., *T. elongata* Ledeb. Their structures have been found out by alkaline hydrolysis and UV, IR, ^{1}H and ^{13}C NMR, COSY-45°, HMQC, HMBC, EIMS, FABMS (-ve) spectral analyses.

Ethanol extracts (70%), chloroform, ethylacetate extracts from *Tamarix laxa* and *Tamarix elongata* showed antioxidant activity. Ethanol extracts (50%) from *Tamarix hispida* contained the triterpenoids which were tested in vitro for inhibiting effect on the α-glycosidase enzyme activity.

3-*α*-[3′′, 4′′-dihydroxy-trans-cinnamoyloxy]-dihydroxy-trans-cinnamoyloxy]-D-friedoolean-14-en-28-oic showed inhibiting effect on the *α*-glycosidase enzyme activity and was found to be a potent antioxidant.

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